COMPOSITIONS AND METHODS FOR TREATMENT OF CRYPTOCOCCOSIS

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods for treating or preventing *Cryptococcus neoformans* infection and conditions caused by such infection. The present invention relates to human antibodies that specifically bind to *C. neoformans* capsular glucuronoxylomannan (GXM) and nucleic acid molecules that encode the antibodies. The invention also relates to isolated heavy and light chain immunoglobulin molecules of the human antibodies to *C. neoformans* GXM. The invention further relates to nucleic acid molecules that encode such heavy and light chain immunoglobulin molecules. The invention further comprises human antibodies to *C. neoformans* GXM that are chimeric, bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to methods of detecting or monitoring *C. neoformans* infection. The invention further relates to methods for making the antibodies in a non-human animal and expressing the antibodies in cell lines including hybridomas and recombinant host cell systems. The invention also relates to kits and pharmaceutical compositions comprising the antibodies. The invention further relates to methods of treating or preventing *C. neoformans* infection and conditions caused by such infection by administering to a patient compositions described herein.

BACKGROUND OF THE INVENTION

[0002] *Cryptococcus neoformans* is an important human pathogen and a major cause of morbidity and mortality in humans, especially those who are immunocompromised.

Despite the availability of antifungal agents active against *C. neoformans*, cryptococcosis is largely incurable in immunocompromised patients because the organism cannot be fully eradicated. Thus, treatment may require costly life-long anti-fungal prophylaxis or maintenance therapy to control the infection.

[0003] Several treatments are available to prevent recrudescent disease, including azole prophylaxis, which along with highly active antiretroviral therapy, has reduced the incidence of HIV-associated cryptococcosis in the developed world. However, cryptococcosis is an emerging problem in other immunocompromised patient populations and remains a major cause of meningoencephalitis in the developing world. Furthermore, because of prolonged maintenance therapies with anti-fungal drugs the incidence of resistant strains is increasing. Accordingly, there is an urgent need for additional approaches for the prevention and treatment of cryptococcosis.

BRIEF SUMMARY OF THE INVENTION

[0004] The present invention provides isolated human antibodies that specifically bind to *C. neoformans* capsular glucuronoxylomannan (GXM). In particular, the invention provides monoclonal antibodies G14F7E5, G15B4G5 and G19B9G7 which recognize *C. neoformans* GXM. Monoclonal antibody G15B4G5 effectively protects against *C. neoformans* challenge in passive immunizations. The invention further provides methods for making the antibodies in non-human animals and by expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. The invention also provides kits and pharmaceutical compositions comprising the antibodies. Moreover, the invention provides methods of treating or preventing *C. neoformans* infection and conditions caused by such infection by administering to a patient pharmaceutical compositions described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Figure 1 shows inhibition curves for G14F7E5, G15B4G5 and G19B9G7 by soluble GXM. Binding by ELISA is shown. Panel A shows inhibition of the binding of Mabs to 24067 by soluble SB4; Panel B shows inhibition of the binding of Mabs to 24067 by 24067; and Panel C shows inhibition of the binding of the Mabs to H99 by soluble SB4. Binding is represented by the absorbance (Abs) at 405 nm as shown on the y axis for concentration of the indicated Mab on the x axis.

[0006] Figure 2 shows a passive immunization experiment with MAbs G14F7E5, G15B4G5 and G19B9G7.

Figure 3 is a table comparing a portion of the CDR1 and the CDR2 of monoclonal antibodies to *C. neoformans* GXM. Residues that are in bold and underlined are ones that are shared by murine and human XenoMouse® mouse-derived monoclonal antibodies; residues in bold and italics are somatic mutations; residues in italics (not bolded) are residues that are similar among antibodies but in different positions; residues in lower case are associated with diminished GXM binding. Hu- human; Ms – mouse.

DETAILED DESCRIPTION OF THE INVENTION

[0007] The present invention provides fully human antibodies or antigen-binding portions thereof that specifically bind to *C. neoformans* GXM. In some embodiments, the fully human antibodies are monoclonal. Other embodiments include nucleic acid molecules comprising nucleotide sequences encoding all or part of the antibodies' heavy and light chains and polypeptides comprising the amino acid sequences encoded by such nucleotide sequences, and in particular sequences comprising the complementarity determining regions (CDRs). Antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein are also provided. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

[0008] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic

acid chemistry and hybridization described herein are those well known and commonly used in the art. Unless otherwise indicated, the methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0009] The following terms are intended to have the following general meanings as they are used herein:

[0010] "B lymphocytic cells or progeny thereof" refer to any cell descending from, or destined for, the B lymphocytic lineage. Examples include, but are not limited to, all B lymphocytes in the B cell developmental pathway starting from the earliest B lymphocyte stem cells through memory B cells, plasma cells, and any hybridomas created *in vitro*.

[0011] "Bispecific antibodies" are single antibodies that have affinities for two separate antigens. For example, a bispecific antibody might recognize *C. neoformans* GXM using one combination of heavy and light chains and might recognize a leukocyte cell surface marker using a second combination of heavy and light chains attached to the first combination. See, e.g., McCormick et al., *J. Immunol.* 158:3474-82 (1997).

[0012] "Chimeric antibodies" are antibodies that have been altered from their original form to comprise amino acid sequences from another protein. Chimeric antibodies retain at least a portion of the original antibody amino acid sequence, typically the portion comprising the antigen binding region (Fab). Examples of chimeric antibodies include, but are not limited to, bispecific antibodies and fusions with other non-immunoglobulin protein sequences.

[0013] "Cytokines" refer generally to signaling molecules of the immune system. Cytokines include, but are not limited to, interleukins (IL), transforming growth factors (TGF), tumor necrosis factors (TNF), lymphotoxins (LT), interferons, granulocyte-macrophage colony stimulating factors (GM-CSF), macrophage CSF, granulocyte CSF, and migration inhibition factors.

[0014] "Derivatize" refers to the process of attaching a non-immunoglobulin agent to the immunoglobulin molecules. Examples of derivatizing agents include, but are not limited to, toxins, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

[0015] "Fusion proteins" refer to chimeric proteins comprising amino acid sequences of two or more different proteins. Typically, fusion proteins result from *in vitro* recombinatory techniques well known in the art. However, fusion proteins may result from *in vivo* crossover or other recombinatory events.

[0016] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, including, e.g., at least 14 amino acids long, at least 20 amino acids long, at least 50 amino acids long, and at least 70 amino acids long.

[0017] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Generally, amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. For example, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. (Bowie et al., *Science* 253:164 (1991)).

[0018] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the

naturally occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., Nature 354:105 (1991), which are each incorporated herein by reference.

[0019] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology - A Synthesis (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ-carboxyglutamate, ϵ-N,N,N-trimethyllysine, ϵ-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, s-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0020] "Human immunoglobulin molecules" refer to immunoglobulin proteins that have a sequence encoded by human immunoglobulin gene sequences. In some embodiments the protein is encoded by the gum-line human gene sequence mother embodiments the protein may comprise mutations from the human germ-line sequence.

[0021] "Human monoclonal antibodies" refer to antibodies that are members of a population of human antibodies with identical specificities. The population of human antibodies may be produced in a hybridoma or other immortalized cell line as well as in recombinant cell lines expressing the exogenous human antibody gene sequences.

[0022] "Immunocompromised patients" refer to patients whose immune responses to foreign antigens or agents is impaired, e.g., by disease (e.g. AIDS), by invasive surgery,

by drug therapies in connection with treatments for other conditions (e.g. organ transplant patients), or due to genetic defects.

[0023] "Toxins" refer to protein or non-protein compounds that can be attached to antibodies for the purpose of killing the cells to which the antibodies have attached. Examples of toxins include, but are not limited to, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

[0024] "Vectors" refer to nucleic acid molecules that allow nucleic acid sequences of interest to be cloned, propagated, recombined, mutated, or expressed outside of their native cells. Often vectors have sequences that allow for controlling expression of gene sequences under specific conditions or in specific cells.

[0025] To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. Expression vectors include plasmids, viruses, retroviruses, cosmids, YACs, EBV-derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0026] A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide

can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0027] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Exemplary regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see, e.g., U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell et al. and U.S. Patent No. 4,968,615 by Schaffner et al.

[0028] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Exemplary selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and the glutamate synthetase gene.

[0029] Nucleic acid molecules encoding anti-GXM antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA

into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, e.g., U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference).

[0030] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[0031] Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

[0032] "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act *in trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to sequences that allow for the inducible or constitutive expression of gene sequences under specific conditions or in specific cells. Examples of cellular processes that expression control sequences regulate include, but are not limited to, gene transcription, protein translation, messenger RNA splicing, immunoglobulin isotype switching, protein glycosylation, protein cleavage, protein secretion, intracellular protein localization and extracellular protein homing. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences

that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence. In eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0033] The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0034] "XenoMouse®" mice refers to mice bearing inactivated endogenous immunoglobulin loci, rendering them incapable of expressing endogenous murine immunoglobulin, but bearing substantial portions of human immunoglobulin loci. Mice of the XenoMouse® line are capable of somatic rearrangement of the human immunoglobulin genes, hypermutation of the human immunoglobulin variable regions, and immunoglobulin isotype switching. Therefore, the mice of the XenoMouse® line are capable of mounting effective humoral responses to antigenic challenge utilizing the human immunoglobulin gene sequences. The resulting antibodies are fully human and can be isolated from the animals themselves, from cultured cells extracted from the animals, or from hybridomas created from XenoMouse® mouse B lymphocytic lines or progeny thereof. Moreover, the rearranged human gene sequences encoding immunoglobulins raised against specific antigenic challenges can be isolated by recombinant means well known in the art.

[0035] *Antibody Structure.* The basic antibody structural unit comprises a tetramer. Each tetramer is composed of two pairs of polypeptide chains, each pair having one

"light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See, generally, *Immunology*, Ch. 4 (Roitt, I., et al., eds., 6th ed., Harcourt Publishers Ltd., London (2001)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0036] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of the Kabat Database of Sequences of Proteins of Immunological Interest (Johnson & Wu, *Nucl. Acids Res.* 29:205-06 (2001); or Chothia & Lesk, *J. Mol. Biol.* 196:901-17 (1987); Chothia et al. *Nature* 342:878-83 (1989)).

[0037] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-21 (1990); Kostelny et al., *J. Immunol.* 148:1547-53 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al., *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-48 (1993)); or "Janusins" (Traunecker et al., *EMBO J.* 10:3655-59 (1991) and Traunecker et al., *Intl. J. Cancer Suppl.* 7:51-52 (1992)). Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

[0038] *Human Antibodies from Non-human Animals.* Antibodies with murine or rat variable and/or constant regions are less useful than human antibodies for certain therapeutic uses. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. To avoid these problems with murine or rat derived antibodies, one can, e.g., develop humanized antibodies or generate fully human antibodies through the introduction of human antibody function into a rodent so that the rodent would produce fully human antibodies.

[0039] The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

[0040] An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) - an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can therefore be expected to provide a substantial advantage in the treatment of chronic or recurring human diseases, such as inflammation, autoimmunity, cancer and bacterial infections, which potentially require repeated antibody administrations.

[0041] One approach toward this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the

mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and selected.

[0042] This general strategy was demonstrated in connection with the generation of the first XenoMouse® animal strains. See Green et al., *Nature Genet.* 7:13-21 (1994). The XenoMouse® animal strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb- and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs were compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human monoclonal antibodies. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization.

[0043] The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce new XenoMouse® mice. See Mendez et al., *Nature Genet.* 15:146-56 (1997) and Green & Jakobovits, *J. Exp. Med.* 188:483-95 (1998) the disclosures of which are hereby incorporated by reference.

[0044] Such an approach is further discussed and delineated in U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, published July 25, 1991, WO 94/02602, published February 3, 1994, WO 96/34096 and WO 96/33735, both published October 31, 1996, WO 98/16654, published April 23, 1998, WO 98/24893, published June 11, 1998, WO 98/50433, published November 12, 1998, WO 99/45031, published September 10, 1999, WO 99/53049, published October 21, 1999, WO 00 09560, published February 24, 2000 and

WO 00/037504, published June 29, 2000. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

[0045] Antibodies in accordance with the present invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the above-mentioned patents, applications, and references.

[0046] Through use of such technology, fully human monoclonal antibodies to *C. neoformans* GXM, or the antigen binding portions thereof, were produced. Essentially, we immunized XenoMouse® lines of mice with *C. neoformans* GXM, recovered spleen and lymph node cells (such as B-cells) from the mice that express *C. neoformans* GXM-specific antibodies, fused such recovered cells with nonsecreting myeloma cells to prepare immortal hybridoma cell lines, and screened hybridoma cell lines to identify those that produce antibodies specific to *C. neoformans* GXM.

[0047] Antibodies in accordance with the present invention also can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used for transformation of a suitable host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, for example, packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by United States Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used in a given instance depends upon the host to be transformed. For example, methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0048] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS/O, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human

hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce antibodies with the desired *C. neoformans* GXM binding properties.

[0049] Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, enhanced expression can be realized by the coamplification expression system utilizing dihydrofolate reductase (DHFR) or the glutamine synthetase gene expression system (the GS system). See, e.g., Kaufman and Sharp, *J. Mol. Biol.* 159:601-21 (1982); European Patent Nos. 0 216 846, 0 256 055, and 0 323 997; and European Patent Application No. 89303964.4.

[0050] Antibodies of the invention also can be produced through the generation of an animal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in animals, e.g., antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

[0051] The invention contemplates an isolated human monoclonal antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM. In some embodiments, the isolated human antibody or antigen-binding portion thereof binds *C. neoformans* GXM and enhances resistance of a subject to *C. neoformans*.

[0052] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM, wherein the antibody or antigen-binding portion thereof prevents or reduces the severity of conditions or disorders caused by *C. neoformans* infection.

[0053] The isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM of the invention may be immunoglobulin G (IgG), IgM, IgE, IgA or IgD. In some embodiments, the IgA may be an IgA1 or IgA2 subtype and the IgG may be an IgG1, IgG2, IgG3 or IgG4 subtype.

[0054] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and is labeled. In a preferred embodiment, the label is a radiolabel, an enzyme label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein or a cytokine.

[0055] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises a kappa light chain. In some embodiments, the variable (V) region of the kappa light chain comprises an amino acid sequences is encoded by a human VκIII DPK22/A27 gene with up to 5 mutations from the germ-line sequence. In some embodiments, the joining (J) region of the kappa light chain comprises an amino acid sequence that is encoded by a human Jκ1 gene. Where the amino acid sequence comprises mutations from the germline V_K and/or J_K sequences, the mutations can be in framework regions, CDR5 or both.

[0056] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises a kappa light chain comprising an amino acid sequence shown in Table 3 (SEQ ID NO: 1; SEQ ID NO: 5; SEQ ID NO: 9) or the variable region thereof. The invention also contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises a kappa light chain comprising the CDR1 and CDR3 amino acid sequences shown for Mabs G14F7E5, G15B4G5 and G19B9G7 in Table 3 (SEQ ID NOs: 2 and 4; SEQ ID NOs: 6 and 8; and SEQ ID NOs: 10 and 12, respectively). In some embodiments, the antibody comprises a kappa light chain comprising the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of an amino acid sequence shown in Table 3 (SEQ ID NO: 1, SEQ ID NO: 5; SEQ ID NO: 9). In some embodiments the antibody comprises a kappa light chain comprising the amino acid sequences shown in SEQ ID NOS: 2-4, 6-8 or 10-12. The invention further contemplates an anti *C. neoformans* GXM antibody comprising the FR1, FR2, FR3 and/or FR4 amino acid sequences in any one of SEQ ID NOS: 1, 5 or 9. The invention further contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises a kappa light chain comprising an amino acid sequence encoded by a nucleic acid sequence shown in Table 2 (SEQ ID NO: 13; SEQ ID NO: 17; or SEQ ID NO: 21) or the variable region of said amino acid sequence. A signal sequence may or may not be present in any of the antibodies of the invention. The invention also contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises a lambda light chain.

[0057] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM, comprising a heavy chain composed of variable (V), diversity (D), and joining (J) regions of the G14F7E5, G15B4G5 or G19B9G7 antibody. The invention contemplates an isolated human

antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises one or more of the CDR1, CDR2 and CDR3 regions from a heavy chain of the G14F7E5, G15B4G5 or G19B9G7 antibody.

[0058] More specifically, the invention contemplates an isolated human antibody or antigen binding portion thereof that specifically binds *C. neoformans* GXM and comprises a heavy chain that comprises an amino acid sequence that is encoded by a human V_H3 family gene or a human V_H6 gene. In some embodiments the human gene is a V_H3-64 or a VH6-1 gene. In antibodies utilizing a V_H3 family gene the heavy chain amino acid sequence is preferably the germline V_H3 sequence although the invention includes human V_H3 utilizing antibodies with up to 3 mutations from the germline sequence. In some embodiments, the heavy chain further comprises an amino acid sequence encoded by a human D3-9 or a human D3-10 gene. In some embodiments the heavy chain further comprises an amino acid sequence encoded by a human J_H4b or J_H5b gene or said sequence with 1 mutation from the germline sequence. In the case of antibodies utilizing a human V_H6-1 gene the antibody sequence can have from 0-6 mutations from germline. Where said amino acid sequences comprise mutations from the germline V_H1, D and/or J_H sequences, the mutations can be in framework regions CDR5 or both.

[0059] In a preferred embodiment, the heavy chain variable region comprises the amino acid sequence encoded by a human V_H3-65 gene, a human D3-9 gene and a human J_H4b gene. In other embodiments, the heavy chain variable region is encoded by a human V_H64 gene, a human D3-10 gene and a human J_H5b gene.

[0060] The invention further provides an anti-*C. neoformans* antibody that comprises the amino acid sequences of the heavy chain CDR1, CDR2 and CDR3 of SEQ ID NO: 43 (G14F7E5), SEQ ID NO: 47 (G15B4G5) or SEQ ID NO: 51 (G19B9G7), the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of any one of said SEQ ID NO:s, or the amino acid sequence of the variable region any of said SEQ ID NO:s.

[0061] The invention further provides an antibody that specifically binds *C. neoformans* GXM wherein said antibody comprises a heavy chain comprising the amino acid sequence of the FR1, F2, F3 and/or F4 of any one of SEQ ID NOS 43, 47 or 51.

[0062] In some embodiments the affinity of anti-GXM antibodies, expressed as the aKa for GXM 24067 is $1 \times 10^3\ M^{-1}$ or greater. In some embodiments, the aKa is $1.3 \times 10^3\ M^{-1}$, $2 \times 10^3\ M^{-1}$ or $2.1 \times 10^3\ M^{-1}$. As used herein, aKa is the inverse of the soluble GXM antigen concentration at 50% maximal binding to solid-phase antigen.

[0063] In some embodiments the anti-GXM antibodies of the invention bind soluble cell surface bound GXM from serotype A *C. neoformans*, for example GXM from strain SB4 and H99.

[0064] In some embodiments, the anti-GXM antibodies of the invention mediate C3 complement deposition.

[0065] In some embodiments the anti-GXM antibodies of the invention show protection against *C. neoformans* infection.

[0066] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and comprises an antigen binding domain chosen from the list consisting of an Fab fragment, an F(ab')2 fragment and an Fv fragment.

[0067] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and the antibody is a single chain antibody.

[0068] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and the antibody is a chimeric antibody. In a preferred embodiment, the chimeric antibody comprises framework regions and CDR regions from different human antibodies. In a more preferred embodiment, the chimeric antibody is bispecific. In a more preferred embodiment, the chimeric antibody is bispecific for *C. neoformans* GXM and a label selected from the list consisting of a radiolabeled molecule, an enzymatic label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein and a cytokine.

[0069] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM wherein the antibody or portion thereof is derivatized. In a preferred embodiment, the antibody or portion thereof is derivatized with polyethylene glycol, at least one methyl or ethyl group or at least one carbohydrate moiety.

[0070] One may use the nucleic acid molecules of the invention to generate antibody derivatives using techniques and methods known to one of ordinary skill in the art.

[0071] In another embodiment, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-GXM antibodies. The antibodies may be mutated in the variable domains of the heavy and/or light chains to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to

increase or decrease the K_d of the antibody for GXM, to increase or decrease K_{off}, or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook et al. and Ausubel et al., *supra*. If it is desired to introduce mutations in the antibodies, preferably the mutations are made at an amino acid residue that is known to be changed compared to germline in a variable region of an anti-GXM antibody. More preferably one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a variable region of one of the anti-GXM antibodies of the invention. In another embodiment, the nucleic acid molecules are mutated in one or more of the framework regions. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-GXM antibody. A mutation in a framework region or constant domain may also be made to alter the immunogenicity of the antibody, to eliminate deanudation sites or glycosylation sites to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation. Mutations may be made in each of the framework regions, the constant domain and the variable regions in a single mutated antibody. Alternatively, mutations may be made in only one of the framework regions, the variable regions or the constant domain in a single mutated antibody.

[0072] In another embodiment, a fusion antibody or immunoadhesin may be made which comprises all or a portion of an anti-GXM antibody linked to another polypeptide. In a preferred embodiment, only the variable regions of the anti-GXM antibody are linked to the polypeptide. In another preferred embodiment, the V_H domain of an anti-GXM antibody is linked to a first polypeptide, while the V_L domain of an anti-GXM antibody is linked to a second polypeptide that associates with the first polypeptide in a manner in which the V_H and V_L domains can interact with one another to form an antibody binding site. In another preferred embodiment, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (see also Single Chain Antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. The fusion antibody is useful to detecting GXM. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody. In one embodiment, the fusion antibody or immunoadhesin is prepared using one or more CDR regions from an anti-GXM antibody.

[0073] The invention contemplates a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM. The invention further contemplates a kit comprising the antibody or antigen-binding portion thereof, a pharmaceutically acceptable carrier therefor, and a container. In some embodiments, the kit further comprises instructions for use.

[0074] The invention contemplates a method for treating or preventing or inhibiting *C. neoformans* infection or lessening the severity of a condition or disorder caused by such infection, comprising the step of administering an antibody of the invention or an antigen-binding portion thereof, or a pharmaceutical composition comprising said antibody or portion to a patient in need thereof, such as a patient who is at risk of being infected with, or currently infected with, *C. neoformans*.

[0075] In some embodiments, the patient is an immunocompromised patient. Immunocompromised patients may be patients whose immune responses is impaired by age, disease or drug treatment, including treatment with immunosuppressing agents or anti-neoplastic or other chemotherapeutic agents. In some embodiments the immunocompromised patient suffers from antibody gene repertoire defects, particularly defects or deficits in the human V_H3 family genes. Patients who may benefit from treatment with an anti-GXM Ab of the invention can be of any age, i.e., infants and children up to elderly patients.

[0076] In a preferred embodiment, the human antibody is obtained from a non-human animal. In a more preferred embodiment, the antibody is a monoclonal antibody. In another preferred embodiment, the pharmaceutical composition is administered via injection, transmucosal, oral, inhalation, ocular, rectal, long-acting implantation, liposomes, emulsion, cream, topical or sustained-release means. In another preferred embodiment, the antibody is a fusion with a second protein. In a more preferred embodiment the second protein is chosen from the list consisting of a toxic peptide moiety, a complement protein, a radiolabeled protein, a cytokine or an antibiotic protein. In another preferred embodiment, the antibody is labeled with a radiolabel, a toxin, a complement protein, a cytokine or an antibiotic. In another preferred embodiment, the pharmaceutical composition further comprises a toxin, complement protein, radiolabeled protein, cytokine, antibiotic, or any combination thereof.

[0077] The invention contemplates an isolated cell that produces a human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM. In some

embodiments, the cell is chosen from the list consisting of a bacterial cell, a yeast cell, an insect cell, an amphibian cell and a mammalian cell. In specific embodiments, the mammalian cell is selected from the list consisting of a human cell, a mouse cell, a rat cell, a dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell. In other specific embodiments, the mammalian cell is selected from the list consisting of a HeLa cell, a NIH 3T3 cell, a CHO cell, a BHK cell, a VERO cell, a CV-1 cell, a NS/0 cell and a COS cell. In other embodiments, the cell line is a hybridoma.

[0078] The invention contemplates a method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM, comprising: a) culturing a non-human cell capable of producing the antibody under conditions in which the antibody is produced; b) isolating the antibody from the cell culture.

[0079] In some embodiments, the method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM utilizes an immortalized cell line. In some embodiments, the immortalized cell line is a hybridoma.

[0080] The invention contemplates the production of additional human antibody or antigen-binding portions thereof that specifically bind to *C. neoformans* GXM, comprising: a) immunizing a non-human animal comprising a human immunoglobulin locus with a *C. neoformans* antigenic composition; b) allowing the non-human animal to mount a humoral response to the antigenic composition; and c) isolating the human antibody from the non-human animal.

[0081] The invention contemplates a nucleic acid molecule isolated from a non-human animal that comprises a nucleotide sequence that encodes a human antibody heavy chain or the portion thereof that specifically binds to *C. neoformans* GXM. In some embodiments, the nucleic acid molecule is isolated from a hybridoma that produces the human antibody.

[0082] The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, comprising a nucleotide sequence encoding a human antibody heavy chain or antigen-binding portion thereof comprising a nucleotide sequence encoding the heavy chain of G14F7E5, G15B4G5 or G19B9G7 Mab, wherein the human antibody specifically binds to *C. neoformans* GXM. In a preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to three of the CDR regions of the human antibody. The invention further contemplates an isolated nucleic acid molecule, or a fragment thereof, comprising a nucleic acid sequence encoding a human

antibody heavy chain or antigen-binding portion thereof comprising a CDR3 amino acid sequence of the G14F7E5, G15B4G5 or G19B9G7 Mab, wherein the human antibody specifically binds to *C. neoformans* GXM.

[0083] The invention contemplates an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a heavy chain and/or a light chain of an anti-*C. neoformans* antibody or an antigen-binding portion thereof.

[0084] In some embodiments, the nucleic acid comprises nucleotide sequences encoding one or more heavy chain or light chain CDR amino acid sequences selected from SEQ ID NOS: 44, 45, 46, 48, 49, 50, 52, 53 or 54. In some embodiments the nucleic acid comprises nucleotide sequences encoding the CDR1, CDR2 and CDR3 amino acid sequence found in any one of SEQ ID NOS: 43, 47 or 51.

[0085] In still other embodiments, the nucleic acid comprises a nucleotide sequence encoding the amino acid sequence of any one of SEQ ID NOS: 43, 47 or 51, or of the variable region portion of said sequence.

[0086] In some embodiments the nucleic acid comprises a nucleotide sequence encoding the amino acid sequence of the FR1, FR2, FR3 and/or FR4 regions of any one of the SEQ ID NOS: 43, 47 or 51.

[0087] In some embodiments, the nucleic acid molecule comprise a nucleotide sequence selected from the group consisting of: SEQ ID NO: 31, SEQ ID NO: 35 and SEQ ID NO: 39, or the nucleotide sequence of the variable region, one or more CDRs and/or one or FRs thereof.

[0088] The invention contemplates a vector comprising a nucleic acid molecule, or fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof, wherein the antibody specifically binds to *C. neoformans*. In a preferred embodiment, the vector further comprises expression control sequences operably linked to the nucleic acid.

[0089] The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, encoding a human antibody light chain or antigen-binding portion thereof comprising a nucleotide sequence as indicated in Table 2 (SEQ ID: 13, SEQ ID:17, or SEQ ID:21), or the nucleotide sequence of the variable region or one or more FR regions thereof, wherein the antibody specifically binds to *C. neoformans* GXM. In a preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to three of the CDR regions of the human antibody. The invention further contemplates an isolated nucleic acid molecule, or a fragment thereof, comprising a

nucleic acid sequence encoding a human antibody light chain or antigen-binding portion thereof comprising the CDR1 and CDR3 amino acid sequence as indicated in Table 3 (SEQ IDs: 2 and 4; SEQ ID NOs: 6 and 8; or SEQ ID NOs: 10 and 12), wherein the human antibody specifically binds to *C. neoformans* GXM. The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, comprising a nucleic acid sequence encoding a human antibody light chain or antigen-binding portion thereof comprising CDR1 and CDR3 amino acid sequences as indicated in Table 2 for Mabs G14F7E5, G15B4G5 or G19B9G7 (SEQ ID NOs: 14 and 16; SEQ ID NOs: 18 and 20; or SEQ ID NOs: 22 and 24, respectively), wherein the human antibody specifically binds to *C. neoformans* GXM.

[0090] The invention contemplates a vector comprising a nucleic acid molecule, or fragment thereof, encoding a human antibody light chain or antigen-binding portion thereof that specifically binds to *C. neoformans*. In a preferred embodiment, the vector further comprises an expression control sequence operably linked to the nucleic acid.

[0091] The invention contemplates an isolated host cell comprising: a) a nucleic acid molecule that was isolated from a non-human animal and encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *C. neoformans* GXM; or b) a vector comprising the nucleic acid molecule.

[0092] The invention contemplates an isolated host cell comprising: a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof of a human antibody that specifically binds to *C. neoformans* GXM; or b) a vector comprising the nucleic acid molecule.

[0093] The invention contemplates an isolated host cell comprising: a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *C. neoformans* GXM; or b) a vector or vectors comprising the nucleic acid molecules.

[0094] The invention contemplates a method of recombinantly producing the heavy chain or the antigen-binding portion thereof, the light chain or the antigen-binding portion thereof, or both the light chain and heavy chain or antigen-binding portions thereof, of a human antibody that was identified from a non-human animal and specifically binds to *C. neoformans* GXM, comprising the step of cultivating the host cells described above under conditions in which the nucleic acid molecules are expressed.

[0095] The invention contemplates an isolated human antibody heavy chain or antigen-binding portion thereof, wherein the antibody specifically binds to *C. neoformans* GXM, encoded by any of the nucleic acid molecules encoding the heavy chain described above, or isolated from any of the host cells described above.

[0096] The invention contemplates an isolated human antibody light chain or antigen-binding portion thereof, wherein the antibody specifically binds to *C. neoformans* GXM, encoded by any of the nucleic acid molecules encoding the heavy chain described above, or isolated from any of the host cells described above.

[0097] The invention contemplates a non-human transgenic animal comprising any of the nucleic acid molecules described above. In a preferred embodiment, the non-human transgenic animal expresses the nucleic acid molecule or molecules. In a more preferred embodiment, the non-human transgenic animal comprises an isolated nucleic acid molecule that encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *C. neoformans* GXM, and the non-human animal expresses both nucleic acid molecules. In a more preferred embodiment, the non-human animal is selected from the list consisting of a mouse, a rat, a hamster, a cow, a sheep, a primate, a horse and a pig. In a more preferred embodiment, a human antibody resulting from expression of the isolated nucleic acid molecules or portions thereof is expressed on the surface of cells derived from the animal's B lymphocytic cells or progeny thereof. In another preferred embodiment, the human antibody resulting from expression of the isolated nucleic acid molecules or a portion thereof is secreted into the lymph, blood, milk, saliva, or ascites of the animal.

[0098] The invention contemplates a fusion protein comprising an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM and a second polypeptide sequence. In various embodiments, the second polypeptide sequence is chosen from the list consisting of an epitope tag, an affinity tag, a toxic polypeptide, an antibiotic, an enzyme, a second antibody sequence, a complement protein, and a cytokine.

[0099] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM, wherein the heavy chain isotype of the antibody is mu, gamma, delta, epsilon or alpha.

[0100] The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *C. neoformans* GXM, wherein the antibody or antigen-binding portion thereof is produced by a process comprising the steps of:

a) immunizing a non-human animal comprising a human immunoglobulin locus with an antigen selected from the group consisting of an *C. neoformans* GXM preparation, a virulent *C. neoformans* cell preparation, an attenuated *C. neoformans* cell preparation, and a killed *C. neoformans* cell preparation; b) allowing the non-human animal to mount an immune response to the antigen; and c) isolating the antibody from the non-human animal.

[0101] The invention contemplates an isolated human antibody or antigen-binding portion thereof isolated from an animal or cell that was free of contaminating human biomaterials such as viruses, enzymes, hormones, cytokines, receptors, receptor ligands, immunoglobulins, complement, nuclear proteins, and cytoplasmic signaling proteins. In particular, the human antibodies of the invention are free of Epstein-Barr virus or retroviruses.

[0102] Pharmaceutical compositions may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0103] Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0104] For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For ocular administration, suspensions in an appropriate saline solution are used as is well known in the art.

[0105] For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained as a

solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0106] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0107] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[0108] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0109] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0110] The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be

presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0111] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0112] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

[0113] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0114] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0115] A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its

solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may be substituted for dextrose.

[0116] Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually with a greater toxicity.

[0117] Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

[0118] Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0119] The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[0120] The isolated human antibody or an antigen-binding portion thereof that specifically binds to *C. neoformans* GXM of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

[0121] The kits of the present invention comprise instructions for utilizing the compositions of the present invention for inhibition, prevention or treatment of *C. neoformans* infections or conditions or disorders caused by such infection. The printed instructions on the kit enable one of skill in the art to utilize the kit for practicing the methods of the present invention.

[0122] The following examples are provided by way of illustration only. They are not intended to limit the scope of the invention disclosed herein.

[0123] Example 1 – Generation of Monoclonal Antibodies to *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan (GXM)

[0124] We vaccinated IgG2-kappa human immunoglobulin transgenic mice (XenoMouse® mice; Mendez et al., *Nat. Genet.* 15, pp. 146-56 (1997)) with glucuronoxylomannan of *C. neoformans* serotype D (Strain 24067, ATCC) conjugated to diphteria toxoid (GXM-DT). Vaccinations were performed subcutaneously at the base of the tail. A 100 μl injection of 10 μg of GXM-DT with 50 μl Alhydrogel and 10 μl CpG was administered to each mouse three times: on days 0, 14 and 28. Splenocytes were isolated from the mice on day 42 and hybridomas were produced by fusion of splenocytes with mouse myeloma cell line NSO according to techniques well known in the art (Pirofski et al., *Infect. Immun.* 63: 3005-14, 1995; Chang et. al. *Infect Immun.* 70:4977-86, 2002). Hybridoma cells were cloned in soft agar and propagated using enriched hybridoma medium.

[0125] Over 500 hybridoma cell lines were screened for secretion of antibodies that reacted with GXM 24067. Supernatants from cells showing growth were incubated with polystyrene ELISA plates (Corning™ Glass Works, Corning, NY) coated with 10 μg/ml GXM 24067 at 37°C for 1 hour; the plates were washed and incubated at 37°C for 1 hour with alkaline-phosphatase (AP) conjugated goat anti-human reagents to IgG, IgM, kappa light chains and a goat anti-mouse reagent specific for lambda light chains (FisherBiotech®, Fisher Scientific™, Pittsburgh, PA). The plates were washed and binding was detected with p-nitrophenyl phosphate substrate (Sigma, St. Louis, MO). Optical densities were measured at 405 nm with an MRX Microplate Reader (Dynatech Laboratories, Chantilly, VA). The negative control was an IgM myeloma antibody (Calbiochem®, San Francisco, CA). GXM-binding hybridomas were tested for binding to various antigens including the GXM-mimotope (P13) as described (Zhang et al., *Infect. Immun.* 65 1158-64, 1997 and Fleuridor, et al., *J. Infect. Dis.* 180: 1526-35 (1999), staphylococcal protein A (SPA; Sigma), DT and BSA, using standard techniques as described in (Russell et al., *Infect. Immun.* 68: 1820-26, 2000, Pirofski et al., *Infect. Immun.* 63: 3005-14, 1995 and Chang et al., *Infect. Immun.* 68:1820-26, 2002).

Example 2 - Characterization of MAbs to cryptococcal GXM

[0126] After repeated screening to test for specificity, three hybridomas cell lines were found to have the highest reactivity with GXM and to be reactive with GXM only. The three human MAbs produced by those hybridomas, G14F7E5, G15B4G5 and G19B9G7, were studied further. All three antibodies are IgM and react with GXM 24067 and did not exhibit significant (above background) binding to DT, BSA or the GXM mimotope P13 by direct ELISA. All three MAbs bound to the GXM on *C. neoformans* cells from strain 24067 based on immunofluorescent staining.

[0127] The three hybridomas were deposited on May 1, 2003 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. The following ATCC accession numbers were assigned:

G14F7E5	PTA-5170
G15B4G5	PTA-5171
G19B9G7	PTA-5172

[0128] The specificity of the MAbs for *C. neoformans* serotype A also was determined. Serotype A is the most common cause of cryptococcosis in North America; whereas serotype D is the second most common serotype in North America and is a common cause of disease in Europe. The serotype specificity studies were performed using purified GXM and whole cell ELISAs as follows.

[0129] Purified GXM ELISA: Serial dilutions of each of the MAbs (beginning at10 μg/ml) were incubated with ELISA plates coated with 10 μg/ml GXM from *C. neoformans* strain 24067, serotype A strains SB4 and H99 (provided by Dr. Arturo Casadevall, Albert Einstein College of Medicine) or P13-dextran in PBS; plates were washed and incubated with a goat anti-human IgM-AP (Fisher) for 1 hr, washed and developed as above. MAbs G14F7E5 and G19B9G7 reacted with both serotype A strains, whereas G15B4G5 should no appreciable binding to either serotype A strain indicating that the MAbs have different strain specificity. The results of this experiment are shown in Table 1.

Table 1: Strain Specificity of Reactivity of Mabs G14F7E5, G15B4G5 and G19B9G7 with soluble GXM.

C. neoformans strain	G14F7E5	G15B4G5	G19B9G7
24067 (serotype D)	+++	+++	+++
SB4 (serotype A)	+++	—	+++
H99 (serotype A)	+++	—	+++

[0130] Whole Cell ELISA: *C. neoformans* strains 24067, SB4 and H99 were grown in Saboraud dextrose broth (Becton Dickinson, Sparks MD) for 2 days, after which the cells were washed in PBS, counted and heat-killed at 68°C for 2 h. Cell death was verified by incubating heat-killed cells in Saboraud dextrose broth overnight at 31°C. The cells were diluted in PBS, plated into ELISA plates at a concentration of $1x10^7$ cfu/ml (50 µl/well) and the plates were incubated overnight at 4°C. As controls a myeloma IgM (Calbiochem) and an anti-PPS8 monoclonal IgM, D11 were used. Unbound cells were removed and bound cells were fixed to the plates by incubation with 150 µl/ well of methanol for 30 min. Plates were washed and blocked with 1% BSA/PBS for 1 h at 37°C. After washing, MAbs or a control myeloma IgM (Calbiochem, San Diego, CA) were added to the plates at an initial concentration of 10 µg/ml, diluted 1:3 and they were incubated for 1 h at 37°C. After washing, the plates were incubated with a 1:1000 dilution (in 0.1% BSA/PBS) of goat anti-human IgM AP-conjugated for 1 h at 37°C. Plates were developed as above and read at a wavelength of 405 nm.

[0131] All three of the MAbs bound to both serotype A strains although mAb G15B4G5 bound to a lesser degree than the other two mAbs.

[0132] Example 3 – Antigen Specificity

[0133] The GXM specificity of the MAbs was confirmed with an inhibition ELISA. As controls, a human myeloma IgM (see above) and D11, which is an IgM specific for serotype 8 of *Streptococcus pneumoniae* (Zhong et al., *Infect. Immun.* 67:4119-27, 1999) were used. ELISA plates, coated with 10 µg/ml of the GXMs (24067 SB4 or H99) or 10 µg/ml of P13-DEX (a dextran conjugate of a previously reported polypeptide GXM-mimetope; Fleuridor et al., *J. Immunol.* 166: 1087-96 (2001); Maitta et al., *Infection Immun.* 72: 196-208 (2004)) for 3 hours at room temperature, washed and blocked with 1% BSA/PBS overnight at 4°C. Plates were incubated with serial dilutions of the soluble

GXMs mixed (beginning with 100 μg/ml) with the MAbs 10 or 1 μg/ml; the plates were washed, incubated with goat-anti-human IgM-AP (Fisher) for 1 hr at 37°C and developed and read as above.

[0134] As shown in Fig. 1, soluble GXM from strain SB4 did not inhibit binding of any of the mAbs to GXM from strain 24067 (panel A).

[0135] Example 4 – Sequence Analysis of MAbs to *C. neoformans* GXM 24067

[0136] We obtained the nucleotide sequences for the light chain variable regions (V_L) of the MAbs by sequencing PCR-amplified V_L cDNA from the hybridomas. We generated V_L cDNA by reverse transcription of RNA using the V_L specific primers: V_K sense, 5'- GAA(CT)ATC(T)GAGCTCACC(GT)CAGTCTCCA-3'(SEQ ID NO: 25; (CT) indicates that position 3 has an equal frequency of being A, C or T; the (T) indicates that position 6 has an equal frequency of being C or T; (GT) indicates that position 15 has an equal frequency of being C, G or T); V_K anti-sense, 5'-CCTGTTGAAGCTCTTTGTGAC-3' (SEQ ID NO: 26). PCR products were sequenced from two independent experiments and were found to be identical. The nucleotide sequences comprising the variable regions and CDRs of G14F7E5, G15B4G5 and G19B9G7 are indicated below in Table 2. The amino acid sequences comprising the variable regions and CDRs of G14F7E5, G15B4G5 and G19B9G7 are shown below in Table 3. We compared the variable-region sequences to the database of human immunoglobulin sequences using DNA PLOT (V Base Index; MRC Center for Protein Engineering, Cambridge, United Kingdom; Mukherjee et al., *J. Exp. Med.* 177:1105-16 (1993)).

Table 2: Nucleic Acid Sequences of Light Chains of Mabs G14F7E5, G15B4G5 and G19B9G7.

Identity of Sequence	Nucleic Acid Sequence	SEQ ID NO:
G14F7E5 variable region	5'- GGCACCCTGT CTTTGTCTCC AGGGGAAAGA GCCACCCTCT CCTGCAGGGC CAGTCAGAGT GTTATCAGCA GCTACTTAGC CTGGTACCAG CAGAAACCTG GCCAGGCTCC CAGGCTCCTC ATCTATGGTG CATCCAGCAG GGCCACTGGC ATCCCAGACA GGTTCAGTGG CAGTGGGTCT GGGACAGACT TCACTCTCAC CATCAGCAGA CTGGAGCCTG AAGATTTTGC AGTGTATTAC TGTCAGCAGT ATGGTAACTC ACGGACGTTC GGCCAAGGGA CCAAGGTGGA AATCAAACGA ACTGTGGCTG CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC CTGACGCTGA GCAAAGCAGA CTACGAAGAA ACACAAAGTT CTACGCCTGC GAAGGTNANN NATCAG –3'	13
G14F7E5 CDR1	5'- AGGGCCAGTC AGAGTGTTAT CAGCAGCTAC TTAGCC –3'	14
G14F7E5 CDR2	5'- GGTGCATCCA GCAGGGCCAC T –3'	15
G14F7E5 CDR3	5'- CAGCAGTATG GTAACTCACG GACG –3'	16
G15B4G5 variable region	5'- CAGTCTCCAG GCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC CTGCAGGGCC AGTCAGAGTG TTAGCAGCAG CTACTTAGCC TGGTACCAGC AGAAACCTGG CCAGGCTCCC AGGCTCCTCA TCTATGGTGC ATCCAGCAGG GCCACTGGCA TCCCAGACAG GTTCAGTGGC AGTGGGTCTG GGACAGACTT CACTCTCACC ATCAGCAGAC TGGAGCCTGA AGATTTTGCA GTGTATTACT GTCAGCAGTA TGGTAGCTCA CGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCCT CAGCAGCACC CTGACGCTGA AGCAAAAGCA	17

Identity of Sequence	Nucleic Acid Sequence	SEQ ID NO:
	GACTACGAAG AAACACAAAG GTTCTACGCC TGCGAANGGT CAANACCAT –3'	
G15B4G5 CDR1	5'- AGGGCCAGTC AGAGTGTTAG CAGCAGCTAC TTAGCC –3'	18
G15B4G5 CDR2	5'- GGTGCATCCA GCAGGGCCAC T –3'	19
G15B4G5 CDR3	5'- CAGCAGTATG GTAGCTCACG GACG –3'	20
G19B9G7 variable region	5'- TCTTTGTCTC CAGGGGAAAG AGCCACCCTC TCCTGCAGGA CCAGTCAGAG TATTACCAAC AGCTACTTAG CCTGGTACCA GCAGAAACCT GGCCAGGCTC CCAGGCTCCT CATCTATGGT GCATCCAGCA GGGCCACTGG CATCCCAGAC AGGTTCAGTG GCAGTGGGTC TGGGACAGAC TTCACTCTCA CCATCAGCAG ACTGGAGCCT GAAGATTTTG CAGTGTATTA CTGTCAGCAG TATGGTAACT CACGGACGTT CGGCCAAGGG ACCAAGGTGG AAATCAAACG AACTGTGGCT GCACCATCTG TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTCACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG AGCAAAGCAG ACTACGAAGA AACACAAAGT TCTACGCCTG CGAAAGGTNN AANANNGATC AAGGG –3'	21
G19B9G7 CDR1	5'- AGGACCAGTC AGAGTATTAC CAACAGCTAC TTAGCC –3'	22
G19B9G7 CDR2	5'- GGTGCATCCA GCAGGGCCAC T –3'	23
G19B9G7 CDR3	5'- CAGCAGTATG GTAACTCACG GACG –3'	24

Table 3: Amino Acid Sequences of Light Chains of Mabs G14F7E5, G15B4G5 and G19B9G7.

Identity of Sequence	Amino Acid Sequence	SEQ ID NO:
G14F7E5 variable region	GTLSLSPGER ATLSCRASQS VISSYLAWYQ QKPGQAPRLL IYGASSRATG IPDRFSGSGS GTDFTLTISR LEPEDFAVYY CQQYGNSRTF GQGTKVEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEE TQSSTPAKVX XQ	1
G14F7E5 CDR1	RASQSVISSY LA	2
G14F7E5 CDR2	GASSRAT	3
G14F7E5 CDR3	QQYGNSRT	4
G15B4G5 variable region	QSPGTLSLSP GERATLSCRA SQSVSSSYLA WYQQKPGQAP RLLIYGASSR ATGIPDRFSG SGSGTDFTLT ISRLEPEDFA VYYCQQYGSS RTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSP QQHPDAEAKA DYEETQRFYA CEXSXP	5
G15B4G5 CDR1	RASQSVSSSY LA	6
G15B4G5 CDR2	GASSRAT	7
G15B4G5 CDR3	QQYGSSRT	8
G19B9G7 variable region	SLSPGERATL SCRTSQSITN SYLAWYQQKP GQAPRLLIYG ASSRATGIPD RFSGSGSGTD FTLTISRLEP EDFAVYYCQQ YGNSRTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEETQS STPAKGXXXI K	9
G19B9G7 CDR1	RTSQSITNSY LA	10
G19B9G7 CDR2	GASSRAT	11
G19B9G7 CDR3	QQYGNSRT	12

[0137] The V_L chain variable region gene transcripts of all three MAbs, utilize a human V_KA27 (DPK22) gene and a human J_K1 light-chain gene element. Sequence comparisons

show that G15B4G5 has a germline A27 sequence. In contrast, both G14F7E5 and G19B9G7 have the same nucleotide substitution in the CDR3 region, G to A (position 257 in SEQ ID NO: 13 and position 248 in SEQ ID NO: 21). G15B4G5, lacks this substitution. This substitution results in an amino acid change from Serine (S) to Asparagine (N) (position 86 in SEQ ID NO: 1 and position 83 in SEQ ID NO: 9). Sequence comparisons show that G14F7E5 has a single nucleotide substitution in CDR1 from G to T (position 65 in SEQ ID NO: 13). This substitution results in an amino acid change from Serine (S) to Isoleucine (I) (position 22 in SEQ ID NO: 1). G19B9G7 has four substitutions in the CDR1: from G to A at positions 40, 52 and 59 in SEQ ID NO: 13, and from G to C at position 56 in SEQ ID NO: 13. These substitutions result in amino acid changes as follows: from Alanine (A) to Threonine (T) at position 14 in SEQ ID NO: 9; from Valine (V) to Isoleucine (I) at position 18 in SEQ ID NO: 9; from Serine (S) to Threonine (T) at position 19 in SEQ ID NO: 9; and from Serine (S) to Asparagine (N) at position 20 in SEQ ID NO: 9.

[0138] We determined the V_H gene sequences from PCR-amplified V_H gene cDNA from the hybridomas producing Mabs G14F7E5, G15B4G5 and G19B9G7 as follows. We generated V_H cDNA by reverse transcription of RNA using V_H constant region primers. We then amplified the V_H region from the cDNA by PCR using a mixture of V_H specific primers which collectively cover all of the V_H genes in the Xenomouse® mice used:

V_H3-07 sense, 5'-CACCATGGARTTGGGGCTGAGCTGG-3' (SEQ ID NO: 29);
V_H3-09 sense, 5'-CACCATGGAGTTKGGACTGAGCTGG-3' (SEQ ID NO: 55);
V_H3-11 sense, 5'-CACCATGGAGTTTGGGCTKAGCTGG-3' (SEQ ID NO: 56);
V_H3-21 sense, 5'-CACCATGGAACTGGGGCTCCGCTGG-3' (SEQ ID NO: 57);
V_H3-48 sense, 5'-CACCATGGAGTTGGGGCTGTGCTGG-3' (SEQ ID NO: 58);
V_H3-53 sense, 5'-CACCATGGAGTTTTGGCTGAGCTGG-3' (SEQ ID NO: 59);
V_H3-64 sense, 5'-CACCATGACGGAGTTTGGGCTGAGC-3' (SEQ ID NO: 60); or
V_H6 sense, 5'-CACCATGTCTGTCTCCTTCCTCATCTT-3' (SEQ ID NO: 61); and
V_H3 anti-sense – IgM ASO, 5'-GTGCTGCTGATGTCAGAGTTG-3' (SEQ ID NO: 30).

We gel purified and cloned the V_H PCR products into the pCR1000 plasmid of the TA cloning system (Invitrogen™, San Diego, CA) according to the manufacturer's instructions. We isolated the plasmid DNA using the Maxi plasmid protocol (Qiagen®, Inc., Chatsworth, CA) and sequenced using the ABI-PRISM Big Dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Torrance, CA). We compared the variable-

region sequences to the database of human immunoglobulin sequences using DNA PLOT (V Base Index; MRC Center for Protein Engineering, Cambridge, United Kingdom; Mukherjee et al., *J. Exp. Med.* 177:1105-16 (1993)) to determine the gene usage and to identify the CDR1, CDR2 and CDR3 sequences.

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Table 4: Nucleic Acid Sequences of Heavy Chains of Mabs G14F7E5, G15B4G5 and G19B9G7.

Identity of Sequence	Nucleic Acid Sequence	SEQ ID NO:
G14F7E5 variable region	5'- GAAGATCTCA CCATGTCTGT CTCCTTCCTC ATCTTCTTGC CCGTACTGGG CCTCCCATGG GGTGTCCTGT CACAGGTACA GCTGCAGCAG TCAGGTCCAG GACTGGTGAA GCCCTCGCAG ACCCTCTCAC TCACCTGTGC CATCTCCGGG GACAGTGTCT CTAGCAACAA TGCTGCTTGG AACTGGATCA GGCAGTCCCC ATCGAGAGGC CTTGAGTGGC TGGGAAGGAC ATACTTCAGG TCCAAGTGGT ATAATGATTA TGCAGTATCT GTGAAAAGTC GAATAACCAT CAACCCAGAC ACATCCAAGA ACCAGTTCTC CCTGCAGCTG AACTCTGTGA CTCCCGAGGA CACGGCTGTG TATTACTGTG CTAGAGAGGG TACTATGATT CGGGGAATTA TAAACTGGTT CGACTCCTGG GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GGGAGTGCAT CCGCCCCAAC CCTTTTCCCC CTCGT –3'	31
G14F7E5 CDR1	5'- GGGGACAGTG TCTCTAGCAA CAATGCTGCT TGGAAC –3'	32
G14F7E5 CDR2	5'- AGGACATACT TCAGGTCCAA GTGGTATAAT GATTATGCAG TATCTGTGAA AAGT –3'	33
G14F7E5 CDR3	5'- GAGGGTACTA TGATTCGGGG AATTATAAAC TGGTTCGACT CC –3'	34
G15B4G5 variable region	5'- CTGAGCTGGT TTCCTGTTGC TATTTTTAAA GGTGTCCAGT GTGAGGTGCA GCTGGTGGAG TCTGGGGAAG GCTTGGTCCA GCCTGGGGGG TCCCTGAGAC TCTCCTGTGC AGCCTCTGGA TTCACCTTCA GTAGCTATGC TATGCACTGG GTCCGCCAGG CTCCAGGGAA GGGACTGGAA TATGTTTCAG CTATTAGTAG TAATGGGGGT AGCACATATT ATGCAGACTC TGTGAAGGGC AGATTCACCA TCTCCAGAGA CAATTCCAAG AACACGCTGT ATCTTCAAAT GGGCAGCCTG AGAGCTGAGG ACATGGCTGT GTATTACTGT GCGAGAGATC ATACGATATT TGGACTGGTT CCTCCGTTGG ACTACTGGGG CCAGGGAACC CTGGTCACCG TCTCCTCAGG GAGTGCATCC GCCCCAACCC TTTTCCCCCT CGTCTCCTGT GAGAATTCCC CGTCGGATAC GAGCAGCGTG GCCGTTGGCT GCCTCGCACA GGACTTCCTT CCCGACTCCA TCACTTTCTC CTGGAAATAC AAGAACAACT CTGAC–3'	35
G15B4G5 CDR1	5'- GGATTCACCT TCAGTAGCTA TGCTATGCAC –3'	36

Identity of Sequence	Nucleic Acid Sequence	SEQ ID NO:
G15B4G5 CDR2	5'- GCTATTAGTA GTAATGGGGG TAGCACATAT TATGCAGACT CTGTGAAGGG C –3'	37
G15B4G5 CDR3	5'- GATCATACGA TATTTGGACT GGTTCCTCCG TTGGACTAC –3'	38
G19B9G7 variable region	5'- ATGTCTGTCT CCTTCCTCAT CTTCTTGCCC GTGCTGGGCC TCCCATGGGG TGTCCTGTCA CAGGTACAGC TGCAGCAGTC AGGTCCAGGA CTGGTGAAGC CCTCGCAGAC CCTCTCACTC ACCTGTGCCA TCTCCGGGGA CAGTGTCTCT AGCAACAATG CTGCTTGGAA CTGGATCAGG CAGTCCCCAT CGAGAGGCCT TGAGTGGCTG GGAAGGACAT ACTACAGGTC CTACTGGTAT AATGATTATG CAGTATCTGT GAAAAGTCGA ATAACCATCA ACCCAGACAC ATCCAAGAAC CAGTTCTCCC TGCAGCTGAA CTCTGTGACT CCCGAGGACA CGGCTGTGTA TTACTGTGCA AGAGAGGGTA CTATGATTCG GGGAATTATA AACTGGTTCG ACTCCTGGGG CCAGGGAACC CTGGTCACCG TCTCCTCAGG GAGTGCATCC GCCCCAACCC–3'	39
G19B9G7 CDR1	5'- GGGGACAGTG TCTCTAGCAA CAATGCTGCT TGGAA C –3'	40
G19B9G7 CDR2	5'- AGGACATACT ACAGGTCCTA CTGGTATAAT GATTATGCAG TATCTGTGAA AAGT –3'	41
G19B9G7 CDR3	5'- GAGGGTACTA TGATTCGGGG AATTATAAAC TGGTTCGACT CC –3'	42

Table 5: Amino Acid Sequences of Heavy Chains of Mabs G14F7E5, G15B4G5 and G19B9G7.

Identity of Sequence	Amino Acid Sequence	SEQ ID NO:
G14F7E5 variable region	**EDLTMSVSFL IFLPVLGLPW GVLS**QVQLQQ SGPGLVKPSQ TLSLTCAISG DSVSSNNAAW NWIRQSPSRG LEWLGRTYFR SKWYNDYAVS VKSRITINPD TSKNQFSLQL NSVTPEDTAV YYCAREGTMI RGIINWFDSW GQGTLVTVSS GSASAPTLFP L	43
G14F7E5 CDR1	GDSVSSNNAA WN	44
G14F7E5 CDR2	RTYFRSKWYN DYAVSVKS	45
G14F7E5 CDR3	EGTMIRGIIN WFDS	46
G15B4G5 variable region	**LSWFPVAIFK GVQC**EVQLVE SGEGLVQPGG SLRLSCAASG FTFSSYAMHW VRQAPGKGLE YVSAISSNGG STYYADSVKG RFTISRDNSK NTLYLQMGSL RAEDMAVYYC ARDHTIFGLV PPLDYWGQGT LVTVSSGSAS APTLFPLVSC ENSPSDTSSV AVGCLAQDFL PDSITFSWKY KNNSD	47
G15B4G5 CDR1	GFTFSSYAMH	48
G15B4G5 CDR2	AISSNGGSTY YADSVKG	49
G15B4G5 CDR3	DHTIFGLVPP LDY	50
G19B9G7 variable region	**MSVSFLIFLP VLGLPWGVLS** QVQLQQSGPG LVKPSQTLSL TCAISGDSVS SNNAAWNWIR QSPSRGLEWL GRTYYRSYWY NDYAVSVKSR ITINPDTSKN QFSLQLNSVT PEDTAVYYCA REGTMIRGII NWFDSWGQGT LVTVSSGSAS APT	51
G19B9G7 CDR1	GDSVSSNNAA WN	52
G19B9G7 CDR2	RTYYRSYWYN DYAVSVKS	53
G19B9G7 CDR3	EGTMIRGIIN WFDS	54

The bolded sequences are signal (leader) sequences.

[0139] Analysis of the heavy chain sequence revealed that G15B4G5 comprises the germline sequence of a human V_H 3-64 gene. The variable domain of G15B4G5 further utilizes a human D3-9 gene and a human J_H4b gene. A comparison of the variable domain sequences of G15B4G5, which confers protection against *C. neoformans* infection

and another human anti-*C. neoformans* GXM which has been said to confer protection shows conserved residues in the CDR2. See Fig. 3.

[0140] Example 5 - Mouse Protection Experiments

[0141] The protective efficacy of the MAbs was evaluated in passive protection experiments in mice. Each MAb or the control myeloma IgM was diluted in sterile PBS and 1000, 100, 50, 5 and 0.5 μg doses were administered intraperitoneally (IP) to each of ten 6-8 week old female BALB/c mice (obtained from NCI) one hour prior to IP infection with $5x10^6$ colony-forming-units (cfu) of *C. neoformans* strain 24067. The injections were 0.1 ml, diluted in PBS, and the fungal inoculum was confirmed by plating on saboraud dextrose agar plates (Fisher). The number of surviving mice was monitored daily. When the control mice died, survival was evaluated statistically with the Kaplan-Meier log-rank survival test. A 100 μg dose of G15B4G5 was protective. No other dose or MAb conferred protection that was greater than that for the controls. G19B9G7 enhanced lethality (relative to PBS) at all concentrations. The results of this experiment are shown in Figure 2.

[0142] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0143] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.